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CeO_2 nanoparticles induce no changes in phenanthrene toxicity to the soil organisms *Porcellionides pruinosus* and *Folsomia candida*

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ABSTRACT

Cerium oxide nanoparticles (CeO_2 NPs) are used as diesel fuel additives to catalyze oxidation. Phenanthrene is a major component of diesel exhaust particles and one of the most common pollutants in the environment. This study aimed at determining the effect of CeO_2 NPs on the toxicity of phenanthrene in Lufa 2.2 standard soil for the isopod *Porcellionides pruinosus* and the springtail *Folsomia candida*. Toxicity tests were performed in the presence of CeO_2 concentrations of 10, 100 or 1000 mg Ce/kg dry soil and compared with results in the absence of CeO2 NPs. CeO_2 NPs had no adverse effects on isopod survival and growth or springtail survival and reproduction. For the isopods, LC50s for the effect of phenanthrene ranged from 110 to 143 mg/kg dry soil, and EC50s from 17.6 to 31.6 mg/kg dry soil. For the springtails, LC50s ranged between 61.5 and 88.3 mg/kg dry soil and EC50s from 52.2 to 76.7 mg/kg dry soil. From this study it may be concluded that CeO_2 NPs have a low toxicity and do not affect toxicity of phenanthrene to isopods and springtails.

1. Introduction

Engineered nanoparticles (NPs) are defined as industrially produced materials in which $>50\%$ of the particles have a size distribution ranging from 1 to 100 nm at least in one of their dimensions (European Commission, 2011). New applications of NPs are constantly being developed, resulting in the introduction to the market of diverse products containing NPs (Roco et al., 2011). Within commercially available NPs, CeO_2 NPs have drawn attention due to their potential use as fuel additive in diesel (Cassee et al., 2011; Johnson and Park, 2012). CeO_2 NPs enhance the oxidation rate and decrease the emission of particulate matter during combustion (Jung et al., 2005). Diesel fuel additives show promising improvements for emission reductions (Park et al., 2008). Nevertheless, the potential release of CeO_2 NPs in combination with other emission products in the environment is still unknown. Also mixture toxicology of CeO_2 NPs and organic pollutants in soil has not been studied so far. Predictions of CeO_2 NPs in soils showed an environmental concentration of 0.016 mg/kg in (20-m) areas next to urban roads in the United Kingdom, after 7 years of deposition and are expected to increase up to 0.04 mg/kg after 12 years of deposition (Johnson and Park, 2012). Adverse effect concentrations, however, have shown to be several orders of magnitude above predicted concentrations in the environment (Batley et al., 2013; Johnson and Park, 2012; Park et al., 2008). Studies have suggested that CeO_2 NPs could work as an antioxidant or free radical scavenger leading therefore to a low toxicity (Colon et al., 2010; Schubert et al., 2006; Xia et al., 2008). By comparing CeO_2 NPs and ZnO NPs, Xia et al. (2008) found different mechanisms of toxicity in cellular responses. While ZnO and TiO_2 generated reactive oxygen species (ROS) leading to cell death, CeO_2 NPs showed a protective response by suppressing ROS production.

CeO_2 NPs are likely to co-exist with polycyclic aromatic hydrocarbons (PAHs), as both are released during the combustion process of diesel fuel (Hendren et al., 2011; Park et al., 2008; Scheepers and Bos, 1992; Tavares Jr. et al., 2004). Nanoparticles have a proportionately very large surface area and this surface can have a high affinity for organic chemical combustion products such as PAHs (Moore, 2006). Few toxicity studies have assessed the effects of the combination of NPs and PAHs (Baun et al., 2008; Cui et al., 2011; Hu et al., 2008; Yang and Watts, 2005). Hu et al. (2008) showed that, fullerene C_{60} decreased the bioavailability of phenanthrene by decreasing the freely dissolved concentration in

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water. Still, phenanthrene sorbed to C_{60} was found to be available to the algae *Raphidocelis subcapitata* (previously known as *Pseudokirchneriella subcapitata)* and the crustacean *Daphnia magna* (Baun et al., 2008).

Phenanthrene is a major component of diesel exhaust particles and therefore a common pollutant in the environment. Phenanthrene concentrations in soil vary between urban and unpolluted rural or forest areas, with urban soils presenting higher levels due to anthropogenic emissions. However, a large variation in phenanthrene concentrations in soils can be found. Phenanthrene concentrations were estimated at ~500 μg/kg in urban soils (Wild and Jones, 1995), ~700 μg/kg in street dust particles in Copenhagen (Johnsen et al., 2006), and only 43 μg/kg in urban areas of Tarragona County (Nadal et al., 2011). In unpolluted areas in the UK and Norway, phenanthrene concentrations ranged between 42 to 54 μg/kg (Nam et al., 2008), while in Tarragona County 7 μg/kg was found (Nadal et al., 2011).

No data on combined effects of CeO_2 NPs and phenanthrene is available in the literature for soil organisms. And for the isopod *Porcellionides pruinosus* no data on the toxicity of phenanthrene is available at all. This study therefore aimed at evaluating the influence of CeO_2 NPs on the toxicity of phenanthrene to two soil organisms, the isopod *P. pruinosus* and the springtail *Folsomia candida*. For that purpose, feeding inhibition and reproduction tests were performed with *P. pruinosus* and *F. candida*, respectively. These organisms were chosen because they represent two common groups of organisms found in terrestrial environments, and they present different routes of exposure. Springtails are mainly exposed to dermal uptake from the pore water (Smit and Van Gestel, 1998), while isopods are mainly exposed by oral uptake of soil particles (Vijver et al., 2006).

Three scenarios are possible for the outcome of mixture toxicity tests with phenanthrene and CeO_2 NPs. In a first scenario, the toxicity of the mixture of phenanthrene and CeO_2 NPs is additive, with CeO_2 NPs showing almost no toxicity from the mixture of phenanthrene and CeO_2 NPs compared to phenanthrene alone. In a second scenario CeO_2 NPs act as a synergist and increase toxicity of phenanthrene. Nanoparticles may act as a Trojan horse by enhancing the transport of phenanthrene to intracellular receptors (Farkas et al., 2012), which could lead to a higher toxicity of the mixture. In the third scenario CeO_2 NPs could act as an antagonist and reduce the toxicity of phenanthrene. This could, for instance, be achieved by binding of phenanthrene to the NPs preventing it from entering the cells (Walker et al., 2012).

2. Materials and methods

2.1. Organisms

2.1.1. *Porcellionides pruinosus*

Specimens of the isopod *P. pruinosus* were collected from an unpolluted field in Coimbra (Portugal). The animals were cultured in plastic boxes (24 × 24 cm^2) containing potting soil at 20 ± 2 °C and 16/8 h photoperiod, and fed with alder (*Alnus glutinosa*) leaves. The animals were kept in lab conditions for at least one month before exposure.

2.1.2. *Folsomia candida*

F. candida ("Berlin strain"; VU University, Amsterdam) was cultured in transparent plastic boxes (250 cc) with a moist bottom of plaster of Paris containing charcoal (plaster of Paris: charcoal 12:1 (w/w)) at 20 ± 1 °C at a light/dark regime of 12/12 h. Toxicity tests were initiated with juveniles of the same age (10–12 days) that were obtained by synchronizing the egg laying of the culture animals, fed with dried baker's yeast (Dr. Oetker, Leeuwarden, The Netherlands).

2.2. Soil and pH measurements

Standard Lufa 2.2 natural soil (LUFA-Speyer 2.2, Germany) was used as test soil. This soil is characterized as sandy loam with an organic carbon content of 2.3 ± 0.2%, pH (0.01 M $CaCl_2$) of 5.6 ± 0.4, cation exchange capacity (CEC) of 10.0 meq/100 g and water-holding capacity (WHC) of 46.5%.

Soil pH_{CaCl2} of the test soils was measured at the beginning of the toxicity tests. Spiked soils (5 ± 0.1 g) were shaken with 25 ml 0.01 M $CaCl_2$ solution for 2 h. After settlement of the particles, the pH of the soil solution was recorded using a Consort P907 meter.

2.3. Test compounds and spiking of the soil

Phenanthrene supplied by Sigma-Aldrich (98% purity) was used. CeO_2 NP powder was manufactured by Antaria with a

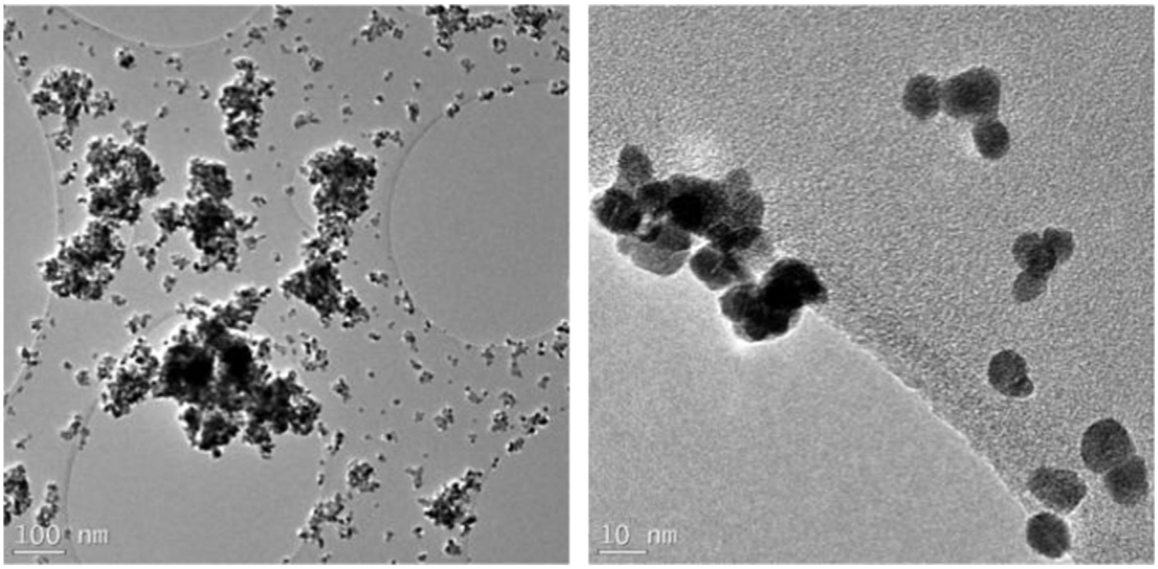

Fig. 1. Transmission Electron Micrographs of CeO_2 NPs, when deposited on a carbon coated Cu TEM grid after dispersion of 1 mg/ml in deionised water and sonicated for 30 s in a low power ultrasonic bath.